Massive recruitment of type I interferon producing plasmacytoid dendritic cells in varicella skin lesions.

Massive Recruitment of Type I Interferon Producing Plasmacytoid Dendritic Cells in Varicella Skin Lesions

Journal of Investigative Dermatology (2006) **126,** 507–509. doi:10.1038/sj.jid.5700052; published online 22 December 2005

TO THE EDITOR

Human plasmacytoid dendritic cells (pDCs) are a subset of leukocytes able to produce large amounts of IFN-α/β and to differentiate into mature dendritic cells in response to viruses (Cella *et al.*, 1999; Siegal *et al.*, 1999). These cells have been identified in the blood, in inflamed secondary lymphoid organs (Facchetti *et al.*, 1988a; Siegal *et al.*, 1999), and in different pathological conditions of the skin (Facchetti *et al.*, 1988a; Jahnsen *et al.*, 2000; Farkas *et al.*, 2001). pDCs can be identified by the co-expression of CD123 and BDCA2 molecules (Dzionek *et al.*, 2001). The capacity of pDCs to produce IFN-α/β depends on the expression of certain molecules called Toll-like receptors (Iwasaki and Medzhitov, 2004). These are cellular sensors able to recognize the presence of DNA and RNA viruses. In particular, pDCs express Toll-like receptor 7, required for recognition of RNA viruses (Diebold *et al.*, 2004; Lund *et al.*, 2004), and Toll-like receptor 9, required for responding to DNA viruses (Krug *et al.*, 2004). For the above features, pDCs are considered important viral detectors and are expected to play a crucial role in antiviral responses (Colonna *et al.*, 2004). However, most of the data on pDCs and viruses are derived from *in vitro* or animal models experiments, while in humans a few studies describe the role of pDCs in virus-related diseases (Soumelis *et al.*, 2001; Kohrgruber *et al.*, 2004; Longman *et al.*, 2005). We investigated pDCs in skin lesions and in the blood of a patient affected by acute varicella infection, and we provide evidence that pDCs may play an important role in the innate immunity responses to viruses *in vivo*.

A 23-year-old man presented with a disseminated maculo-papular and vesicular eruption. Clinically, the diagnosis of varicella (chicken pox) was made, later confirmed by the presence of IgM antibodies against varicella zoster virus. The patient was treated with systemic acyclovir and antihistamines. At the time of the first visit, skin and blood samples were taken after written informed consent. An additional blood sample was obtained 3 weeks later. The study was conducted according to the Declaration of Helsinki Principles. The medical ethical committee of the University of Florence approved all described studies.

Immunohistochemistry analysis of varicella serial sections revealed a high number of CD123+ cells in the dermal infiltrate (Figure 1a and d). The vessels showed intense dilatation and contained numerous CD123+ cells (Figure 1a). Using mAb anti-BDCA2, we observed numerous positive cells co-localizing in the same areas of the CD123+ cells (Figure 1b and e). Within the lumen of the vessels, many BDCA2+ cells appeared adherent to the endothelial wall (Figure 1g). Morphologically, pDCs exhibited a roundish, plasmacytoid appearance (Figure 1d and e) similar to that observed in tonsils (Figure 1h). In normal skin, some scattered BDCA2+ cells were also observed within the papillary dermis (data not shown).

To verify IFN-α/β production by pDCs, we performed immunohistochemistry using anti-MxA mAb, an IFN-α/β-inducible intracellular protein well established as a surrogate marker for local type I IFN production (Fah *et al.*, 1995; Jahnsen *et al.*, 2000). Although MxA expression was not detected in normal skin, an impressive epidermal and dermal overexpression was observed in varicella lesions (Figure 1c). A high number of MxA+ cells showed plasmacytoid morphology and were located in the same areas where CD123/BDCA2-expressing cells were observed, strongly suggesting that pDCs represented the main source of IFN-α/β (Figure 1f).

Recently, the chemokine receptor CXCR3 has been shown to mediate the chemotaxis of human pDCs in virus-induced skin lesions (Kohrgruber *et al.*, 2004); therefore, we stained varicella sections with anti-CXCR3 mAb. Consistent with the previous report, numerous clusters of CXCR3+ cells were observed in the dermal infiltrate, often within the vessels (Figure 1i and j). However, expression of CXCR3 was less strong compared to CD123 and BDCA2 molecules, presumably because pDCs rapidly downregulate CXCR3 upon exposure to viruses (Kohrgruber *et al.*, 2004).

In systemic lupus erythematosus, pDCs accumulate in the skin lesions whereas the number of blood pDCs is decreased (Cederblad *et al.*, 1998; Farkas *et al.*, 2001). Similarly, in the acute phase of varicella infection, flow cytometry analysis showed that blood BDCA2/CD123+ pDCs were barely detectable (0.01% of the total peripheral blood mononuclear cells), in contrast to pDCs of healthy donors (mean ± standard deviation: 0.48% ± 0.13; $N = 4$) (data not shown). Blood examination performed in the patient 3 weeks later showed a considerable increase in the percentage of pDCs (0.68%).

The data presented herein, although limited to a single case, are consistent with the hypothesis that pDCs play an important role in viral skin infections in

Abbreviations: pDC, plasmacytoid dendritic cell

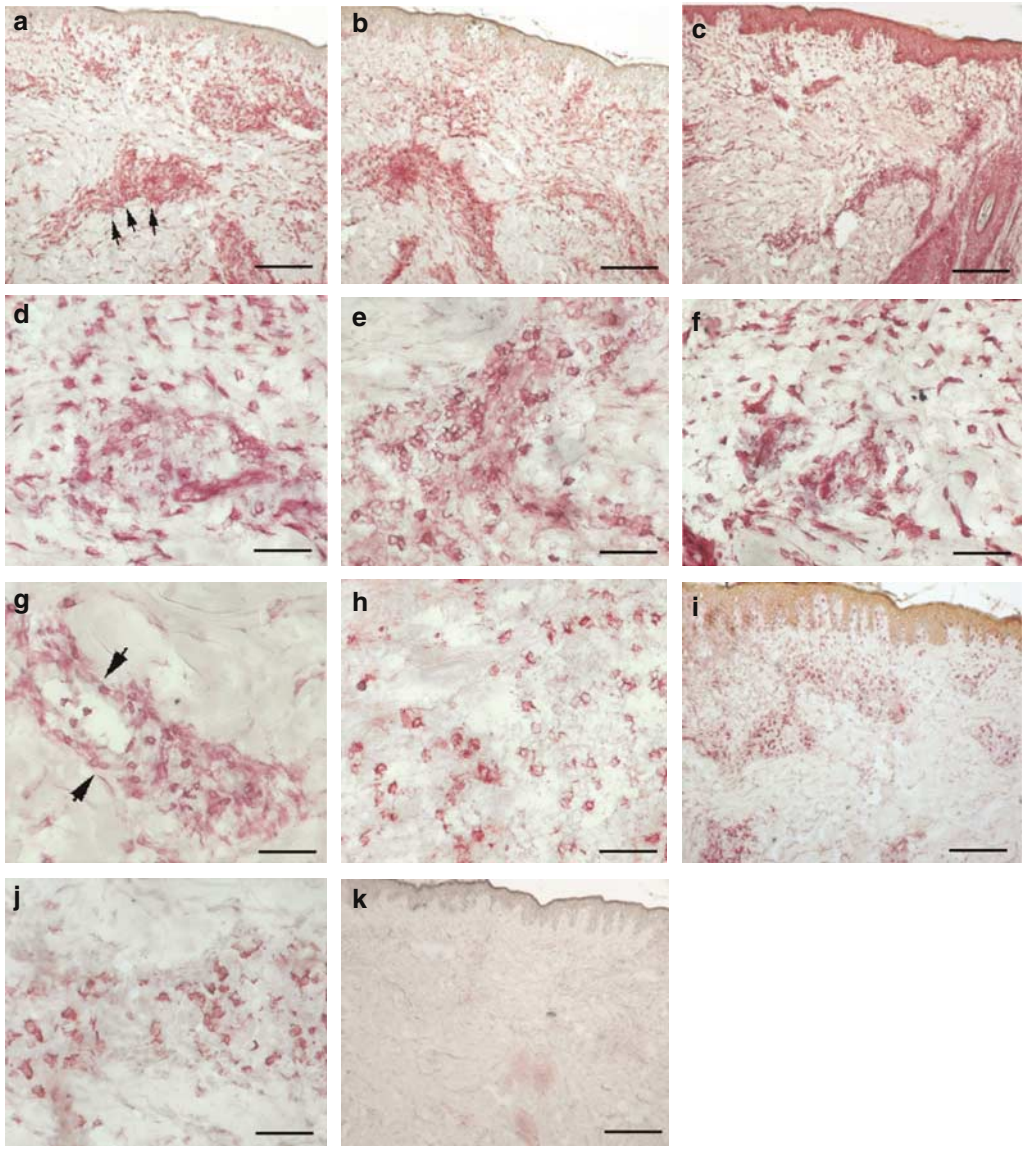

Figure 1. Plasmacytoid dendritic cells infiltrate varicella skin lesions and produce IFN-α/β. Representative cryostat serial sections of a varicella skin lesion were stained with anti-CD123, anti-BDCA2, anti-MxA, and anti-CXCR3 antibodies according to the alkaline phosphatase antialkaline phosphatase technique. The biopsy was performed at the edge of a recently developed vesicle. Samples of normal human skin from the cryomaterial archive of the Department of Dermatological Sciences of Florence were used as negative controls. Tonsils were used as positive control for BDCA2 + pDC. (**a**, **d**) Numerous cells, in the dermal infiltrate and within dilated vessels, are strongly stained by the monoclonal antibody against CD123, which also stains endothelial cells indicated by the arrows (**a**). At higher magnification, most of the CD123-positive cells exhibit a roundish shape (**d**). (**b**, **e**, **g**) A high number of cells stained positive for the BDCA2 molecule. BDCA2 expressing cells co-localize in the same areas of CD123-positive cells in the dermal infiltrate and within the vessels (**b**, **e**). BDCA2-expressing cells show a typical plasmacytoid morphology (**e**) and are found in large numbers within the vessels often adherent to the endothelial wall as indicated by the arrows (**g**). (**c**, **f**) Intense IFN-α/β activity is shown in varicella lesion by staining with an antibody against MxA. (**h**) Sections of tonsils were used as control for BDCA2. (**i**, **j**) Numerous CXCR3-expressing cells are shown in the dermal infiltrate often within the vessels. (**k**) Varicella section stained with an irrelevant anti-idiotype antibody for negative control. Bars = 100 μM (**a**, **b**, **c**, **i**, **k**), 30 μM (**d**, **e**, **f**, **g**, **h**, **j**).

humans, and particularly in the early antiviral responses. Indeed, the patient was observed during an early phase of the varicella eruption when pDC traffic into virally infected sites is expected to be maximal (Kohrgruber *et al.*, 2004). The finding of a high number of CXCR3 + pDC in the dermal infiltrate suggests that these cells might be very rapidly recruited from the blood to the site of virus replication where CXCR3 ligands are strongly produced (Kohrgruber *et al.*, 2004). Consistently, pDC were barely detected in peripheral blood during the acute infection as opposed to healthy individuals. This hypothesis is also supported by the high number of BDCA2-expressing cells observed within the lumen of the vessels and adherent to the endothelial wall, presumably going to enter the dermis. However, the low number of blood pDC observed may also reflect an immunosuppressive

condition, although transient, as described in HIV patients (Soumelis *et al.*, 2001).

A crucial point is that pDCs were functionally active *in situ* as shown by the massive MxA expression in the skin lesion. IFN-α/β secreted by pDCs *in situ* activate natural killer cell activity and, at the same time, protect uninfected cells from natural killer cell-mediated lysis, thus limiting virus replication (Trinchieri *et al.*, 1978).

In conclusion, we have shown in a case report form that pDCs promptly infiltrate varicella skin lesions and are able to produce IFN-α/β *in vivo*. Although these findings need to be extended to a larger number of cases, they represent direct evidence of the importance of pDCs in the early antiviral immune response.

Gianni Gerlini[1], Giulia Mariotti[1], Beatrice Bianchi[1] and Nicola Pimpinelli[1]

[1]Department of Dermatological Sciences, University of Florence Medical School, Florence, Italy. E-mail: g.gerlini@dfc.unifi.it

REFERENCES

Cederblad B, Blomberg S, Vallin H, Perers A, Alm GV, Ronnblom L (1998) Patients with systemic lupus erythematosus have reduced numbers of circulating natural interferon-alpha-producing cells. *J Autoimmun* 11:465–70

Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A *et al.* (1999) Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med* 5:919–23

Colonna M, Trinchieri G, Liu YJ (2004) Plasmacytoid dendritic cells in immunity. *Nat Immunol* 5:1219–26

Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C (2004) Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* 303: 1529–31

Dzionek A, Sohma Y, Nagafune J, Cella M, Colonna M, Facchetti F *et al.* (2001) BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction. *J Exp Med* 194: 1823–34

Facchetti F, De Wolf-Peeters C, van den Oord JJ, Desmet VJ (1988a) Plasmacytoid T cells in a case of lymphocytic infiltration of skin. A component of the skin-associated lymphoid tissue? *J Pathol* 155:295–300

Facchetti F, De Wolf-Peeters C, van den Oord JJ, De vos R, Desmet VJ (1988b) Plasmacytoid T cells: a cell population normally present in the reactive lymph node. An immunohistochemical and electronmicroscopic study. *Hum Pathol* 19:1085–92

Fah J, Pavlovic J, Burg G (1995) Expression of MxA protein in inflammatory dermatoses. *J Histochem Cytochem* 43:47–52

Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL (2001) Plasmacytoid dendritic cells (natural interferon-alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions. *Am J Pathol* 159: 237–43

Iwasaki A, Medzhitov R (2004) Toll-like receptor control of the adaptive immune responses. *Nat Immunol* 5:987–95

Jahnsen FL, Lund-Johansen F, Dunne JF, Farkas L, Haye R, Brandtzaeg P (2000) Experimentally induced recruitment of plasmacytoid (CD123high) dendritic cells in human nasal allergy. *J Immunol* 165:4062–8

Kohrgruber N, Groger M, Meraner P, Kriehuber E, Petzelbauer P, Brandt S *et al.* (2004) Plasmacytoid dendritic cell recruitment by immobilized CXCR3 ligands. *J Immunol* 173: 6592–602

Krug A, Luker GD, Barchet W, Leib DA, Akira S, Colonna M (2004) Herpes simplex virus type 1 activates murine natural interferon-producing cells through toll-like receptor 9. *Blood* 103:1433–7

Longman RS, Talal AH, Jacobson IM, Rice CM, Albert ML (2005) Normal functional capacity in circulating myeloid and plasmacytoid dendritic cells in patients with chronic hepatitis C. *J Infect Dis* 192:497–503

Lund JM, Alexopoulou L, Sato A, Karow M, Adams NC, Gale NW *et al.* (2004) Recognition of single-stranded RNA viruses by Toll-like receptor 7. *Proc Natl Acad Sci USA* 101: 5598–603

Siegal FP, Kadowaki N, Shodell M, Fitzgerald-Bocarsly PA, Shah K, Ho S *et al.* (1999) The nature of the principal type 1 interferon-producing cells in human blood. *Science* 284:1835–7

Soumelis V, Scott I, Gheyas F, Bouhour D, Cozon G, Cotte L *et al.* (2001) Depletion of circulating natural type 1 interferon-producing cells in HIV-infected AIDS patients. *Blood* 98:906–12

Trinchieri G, Santoli D, Dee RR, Knowles BB (1978) Anti-viral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Identification of the anti-viral activity as interferon and characterization of the human effector lymphocyte subpopulation. *J Exp Med* 147: 1299–313